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The impact of *Phaeomoniella chlamydospora* infection on the grapevine's physiological response to water stress Part 1: Zinfandel

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Summary. *Phaeomoniella chlamydospora* is a vascular pathogen that colonises the xylem tissues of the grapevine. It is associated with Petri disease, which is often considered to be 'stress-related'. In glasshouse experiments using Zinfandel, stomatal conductance was higher in infected plants, implying that infection interferes with stomatal control. Leaf water potentials were lower in infected plants subjected to water stress, indicating that infection made it more difficult for the vine to get water to the leaf. Clearly, infection alters the grapevine's physiological response to water stress.

Key words: vascular pathogen, esca, Petri disease, stomatal regulation, leaf water potential.

Introduction

Grapevine trunk diseases limit the long term sustainability of winegrape production in Australia. Petri disease and esca are caused by the xylem-inhabiting fungal pathogen, *Phaeomoniella chlamydospora*, although other fungi have been implicated (Crous and Gams, 2000). Of the two diseases, Petri disease is more prevalent in Australia (Edwards and Pascoe, 2004), primarily affecting young grapevines. Symptoms include graft failure, shoot dieback, slow decline and gradual death of the grapevine (Ferreira *et al.,* 1994) and internal black wood streaking, evident when the trunk of an infected grapevine is cut open. Grapevines can be infected with *P. chlamydospora* yet show no external symptoms of disease (Bertelli *et al.,* 1998; Edwards *et al.,* 2001; Halleen *et al.*, 2003; Edwards and Pascoe, 2004). Infected grapevines are more vulnerable to stress, however, and this can trigger disease expression (Ferreira *et al.,* 1999; Fourie and Halleen, 2004). This is particularly evident during the first few seasons of growth before the grapevine has a well-established root system (Gubler *et al.,* 2004; 2006).

The role of water stress in the development of disease has been examined for several pathosystems, such as Botryosphaeria blight of pistachio (Ma *et al.,* 2001), *Sphaeropsis sapinea* and pine (Blodgett *et al.,* 1997a; 1997b), Cytospora canker of aspen (Guyon *et al.*, 1996), and Pierce's disease of grapevine (Goodwin *et al.,* 1988). Several review papers explore the subject in some detail (Schoeneweiss, 1975, 1986; Boyer, 1995). However, to our knowledge, apart from the research of Ferreira *et*

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al. (1999), there is a lack of published information regarding the effect of water stress on the pathosystem *P. chlamydospora* and grapevine. In Australian viticulture, currently, the trend is for increased promotion and adoption of deficit irrigation schedules to both improve grape quality and conserve water. Given that many Australian grapevines could be harbouring symptomless infections of *P. chlamydospora* (Edwards and Pascoe, 2004), the research presented here aimed to examine the impacts of infection on the grapevine's physiological responses to water deficit.

Materials and methods

Year 1, three-year-old Zinfandel, 12 February – 14 March 2004

A glasshouse experiment was established using three year old potted Zinfandel grapevines which had been propagated from infected mother vines and were known to be naturally infected with *P. chlamydospora*. The uninfected grapevines used in this experiment had been propagated from hot water treated cuttings from the same mother vines. The vines were grown in standard potting mix in 30 cm diameter pots. The plants were regularly pruned to keep the canopy size to approximately 1 m^3. At the end of the trial period, all the vines were destructively assessed to confirm their infection status.

There were four treatments in a 2×2 factorial experiment design, with six replicates per treatment: (1) no water stress, no infection; (2) no water stress, *P. chlamydospora* infection; (3) 50% water deficit, no infection; (4) 50% water deficit, *P. chlamydospora* infection.

The vines were watered daily at 10 am with a measured amount of water. Each day, the mean water use of the vines with no water stress and no infection (treatment 1) was calculated as follows and determined to be the required water for the unstressed treatments. At 10 am on Day A, the six pots from treatment 1 were fully watered, allowed to drain for one hour, then weighed. 24 hours later (Day B), the pots were weighed again prior to watering. The water use per vine was calculated as Day A Pot Weight – Day B Pot Weight. The stressed treatments received 50% of this amount. The treatments were applied for three weeks.

Measures of leaf water potential, stomatal conductance and leaf temperature were made per vine on three days per week (see Table 1). Leaf water potential (ψ_L) was measured destructively using a pressure bomb on one leaf per vine at approximately 3 pm. Using a steady state porometer, stomatal conductance (g_L) was measured between 1–2 pm on three leaves per vine taken from three different positions and averaged over the three leaves. The leaf temperature of five leaves per vine was taken using an infra-red thermometer at approximately 1 pm and averaged over the five leaves. In addition, diurnal leaf water potential measurements were made at 6 am, 9 am, 12 noon, 3 pm, 6 pm on Wednesday of each of the three weeks.

At the end of the experiment, the dry weight of the vines was measured as two variables, the above ground dry weight and the below ground dry

Table 1. Measurements of water stress (WS) in experimental grapevines, years 1 and 2.

WS parameter	Time of measurement	Measures per vine
Leaf temperature (°C)	1–2 pm, ×3/week Mon/Wed/Fri (Yrs 1&2)	5 leaves, mature sunlit, midshoot
Daily stomatal conductance (g_L)	1–2 pm, ×3/week Mon/Wed/Fri (Yr 1) 3–4 pm, ×3/week Mon/Wed/Fri (Yr 2)	3 leaves, mature sunlit, midshoot
Diurnal stomatal conductance (g_L)	6 am, 3 pm, 6 pm; days 7, 9, 13 (Yr 2)	1 leaf, mature sunlit, midshoot
Daily leaf water potential (ψ_L)	3–4 pm, ×3/week Mon/Wed/Fri (Yrs 1&2)	1 leaf, mature sunlit, midshoot
Diurnal leaf water potential (ψ_L)	6 am, 9 am, 12 noon, 3 pm, 6 pm; ×1/week – Wednesdays (Yr 1) 6 am, 3 pm, 6 pm; days 7, 9, 13 (Yr 2)	1 leaf, mature sunlit, midshoot

weight. In addition at the end of the experiment, the infection 'status' of the vines was checked by surface sterilising, cutting and moist incubating the grapevine stems for 4–6 weeks, followed by microscopic examination for *P. chlamydospora*.

The diurnal data were analysed using a standard split-plot analysis. The daily measurements data were analysed using the restricted maximum likelihood (REML) methods of Genstat (Payne, 2005) because not all treatment combinations were present in equal numbers due to the infection status of some vines being revised. A log transformation was required for the conductance data prior to analysis. A linear mixed model was fitted to each variable. The fixed effects model included terms for stress, infection and date and their interactions. The random effects part of the model included terms for the design structure (i.e. terms for replicates and pots). Correlation structures to account for the unequal distances between measurement dates and to allow for unequal variances between measurement points were included.

Year 2, four-year-old Zinfandel, 28 February – 11 April 2005

The experiment was repeated again in the following year, but with a gradually increasing water stress (Table 2) instead of a fixed 50% water stress.

Once again, there were four treatments in a 2×2 factorial experiment design, with six replicates per treatment: (1) no infection, no water stress; (2) *P. chlamydospora* infection, no water stress; (3) no infection, water stress; (4) *P. chlamydospora* infection, water stress.

The vines were watered daily with a measured amount of water, calculated in the same manner as described for Year 1. The stressed treatments received 50% of mean water use in week 1 and 25% in week 2. In week 3, water was completely withheld from the stressed treatments, but after three days it was clear that the vines were shutting down and would die, so all vines received 100% water. Upon recovery (four days), the stress regime was re-introduced as before, beginning with 50% of mean water use for the first week, 25% for the second and third weeks, returning to 100% before the final data measurements. The treatments were applied from 2 March–9 April.

Daily water use, leaf water potential (ψ_L), stomatal conductance (g_L) and leaf temperature measurements were made per vine as described in Table 1. In addition, diurnal stomatal conductance and leaf water potential measurements were made on days 7, 9 and 13 of the experiment at 6 am, 3 pm and 6 pm.

Data were analysed as for year 1.

Results

Year 1, three-year-old Zinfandel, 12 February – 14 March 2004

As expected, stomatal conductance was much lower in the stressed plants than in the unstressed plants (Fig. 1). Although the main effect of infection was not significant at $P<0.05$, stomatal conductance was generally higher in the infected vines than in the uninfected vines (Fig. 1).

For leaf water potential, the main effects of stress, infection and date were significant and there

Table 2. Watering schedule for potted Zinfandel grapevines in Year 2, 2005.

Date	Treatments
28 February	All vines received 100% water
2 March	Stress treatments applied: 50% water
9 March	Stress increased to 25% water; diurnal measurements taken
11 March	Diurnal measurements taken
15 March	Diurnal measurements taken
16 March	Water withheld from stress treatments
19 March	All vines fully watered and allowed to recover
22 March	Stress treatments applied: 50% water
29 March	Stress treatments applied: 25% water
9 April	Full water resumed for all vines
11 April	Final measurements taken

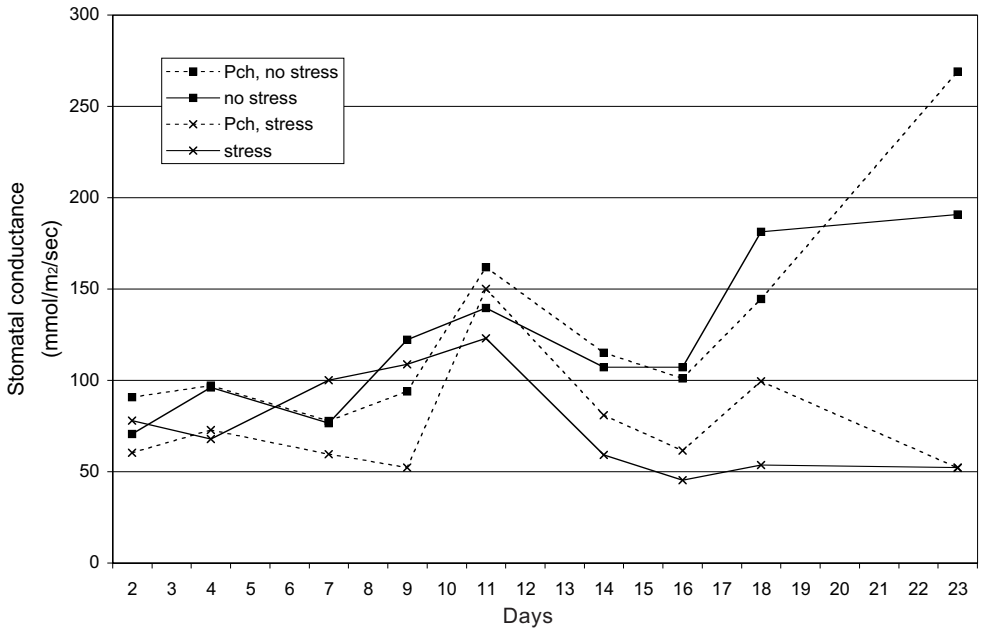

Fig. 1. Effects of water stress on stomatal conductance of infected and uninfected Zinfandel, Year 1: 2004.

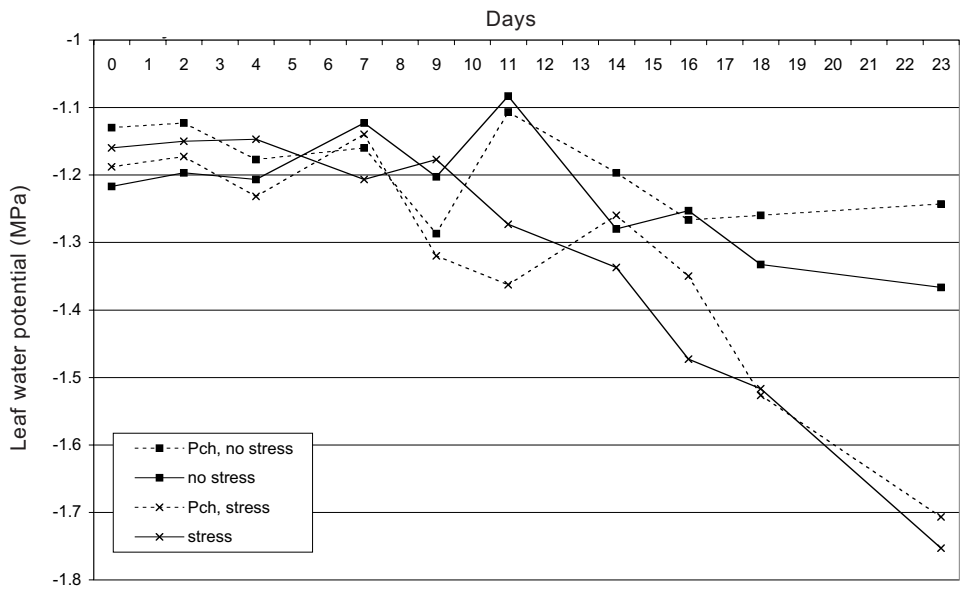

Fig. 2. Effects of water stress on leaf water potential of infected and uninfected Zinfandel, Year 1, 2004.

was a significant interaction (P=0.049) between stress and infection. Leaf water potential was much lower in the stressed plants than the unstressed plants, as expected (Fig. 2).

The diurnal measurements of leaf water potential were the most informative (Fig. 3). In Week 1, there was little difference between how the plants responded until the last measurement of the day, when infected plants were clearly more stressed than uninfected plants. By Week 2, although all the plants were able to recover overnight, by mid-afternoon the plants receiving less water were obviously stressed, and the infected plants were more stressed than their uninfected counterparts. By Week 3, the impact of infection was masked by the significant impact of three weeks of water stress,

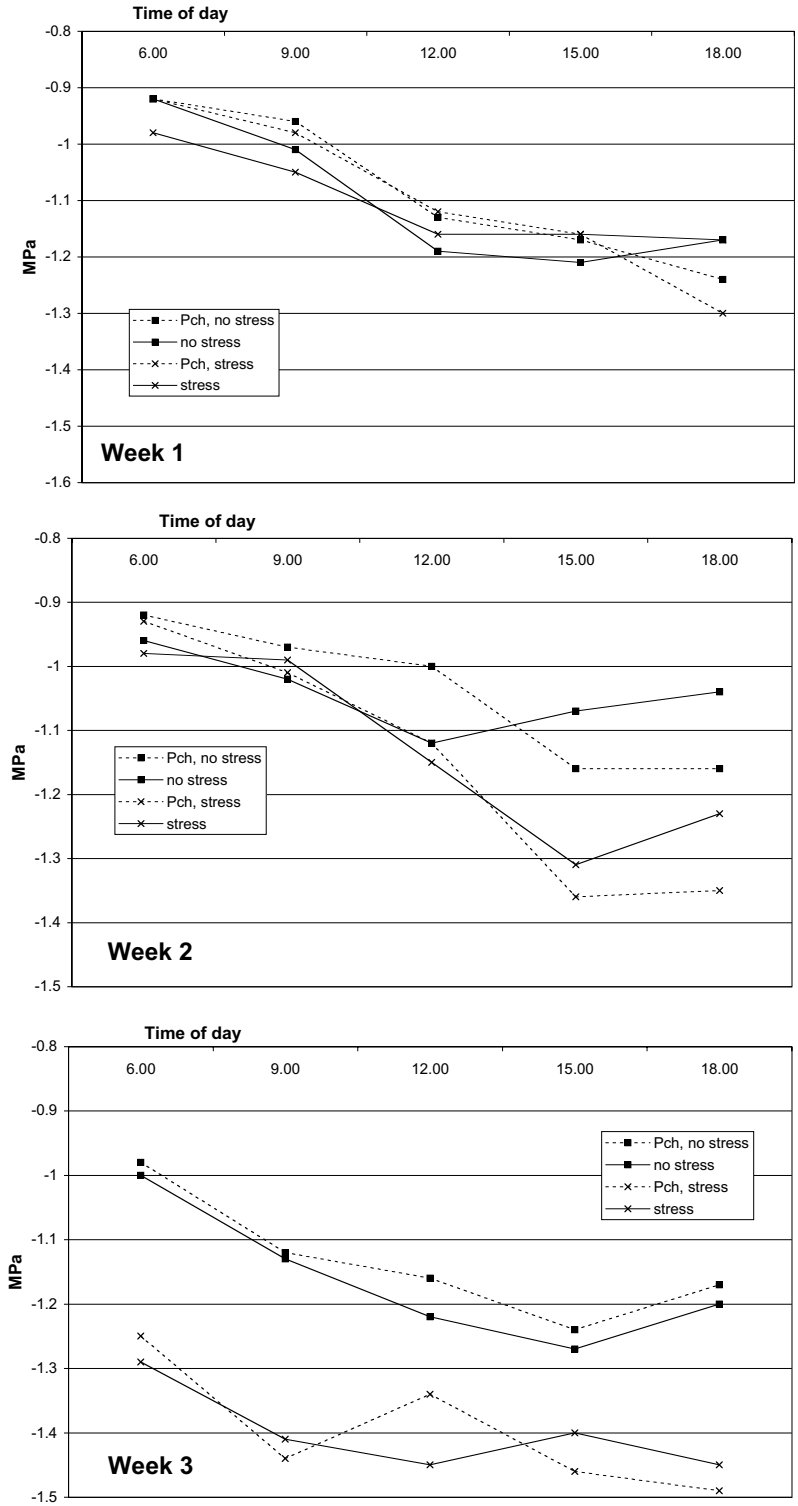

Fig. 3. Effects of water stress on diurnal measurements of leaf water potential of infected and uninfected Zinfandel grapevines (Year 1, 2004, weeks 1, 2, 3).

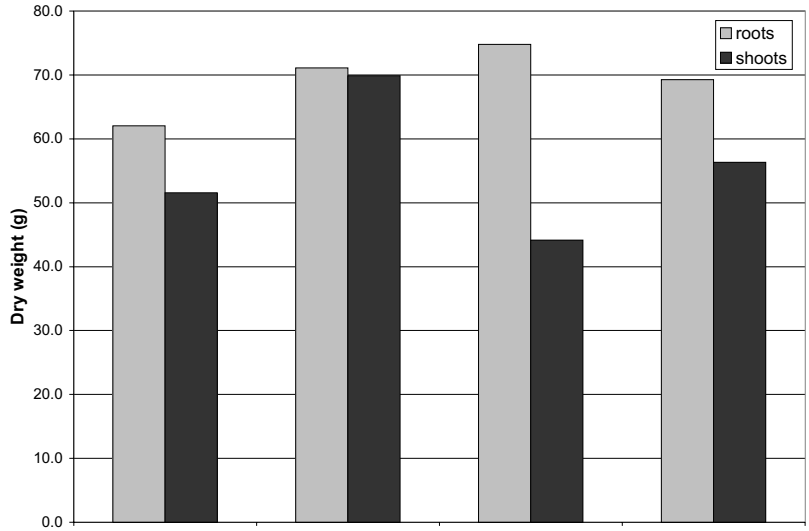

Fig. 4. Effect of water stress on biomass of infected and uninfected Zinfandel, Year 1, 2004.

demonstrated by the fact that the plants did not recover overnight.

Leaf temperature measurements did not differ between treatments (data not shown). There was no significant difference between treatments for root dry weight, but the mean shoot dry weight for the no stress/no infection treatment combination was significantly higher than for the other three treatment combinations (Fig. 4). This confirms that infection and water stress, either alone or in combination, reduce vine growth.

Year 2, four-year-old Zinfandel, 28 February – 11 April 2005.

The overall main effects of date, stress and infection were significant for the daily measurements of stomatal conductance. The stomatal conductance for the infected plants was consistently higher than for the uninfected plants in the unstressed treatment (Fig. 5), and this was supported by a significant interaction between stress and infection ($P<0.001$).

The diurnal measurements of stomatal conductance on days 7, 9 and 13 clearly showed differences between infected and uninfected plants (Fig. 6). The three times of day were analysed as separate variables using analysis of variance. The analysis showed large main effects of stress and date. The interaction of both stress and infection with date showed that these effects changed significantly over the three days at 6 am. There was no interaction between infection and stress at 6 am. At 3 pm, the hottest part of the day, there were significant overall main effects of date and stress, and evidence of an overall infection effect ($P=0.054$). The interaction of stress and date was significant and there was also very weak evidence of an overall interaction between stress and infection ($P=0.088$). This was probably not due to a change in the difference in the effects of infection and stress over time but rather that there was almost no difference between all unstressed vines at 3 pm on day 9 due to dense cloud cover. There was a difference on both day 7 and day 13. At 6 pm, only date and stress effects were significant indicating that on average, infected and uninfected vines were behaving similarly.

As expected, the analysis showed that daily leaf water potentials were lower for stressed plants. There was also a significant main effect of infection but no evidence of an interaction between infection and stress. The significant interaction between date and infection is evidence that daily leaf water potential was lower in the infected stressed plants than in the uninfected stressed plants, and this difference became more pronounced as the stress was prolonged (Fig. 7). The diurnal measurements supported this (Fig. 8). At 6 am, only stress and date were significant suggesting that there was little effect of infection in early morning. However, at 3 pm, infection, stress and date were significant main effects and the interactions of date with both stress and infection were also

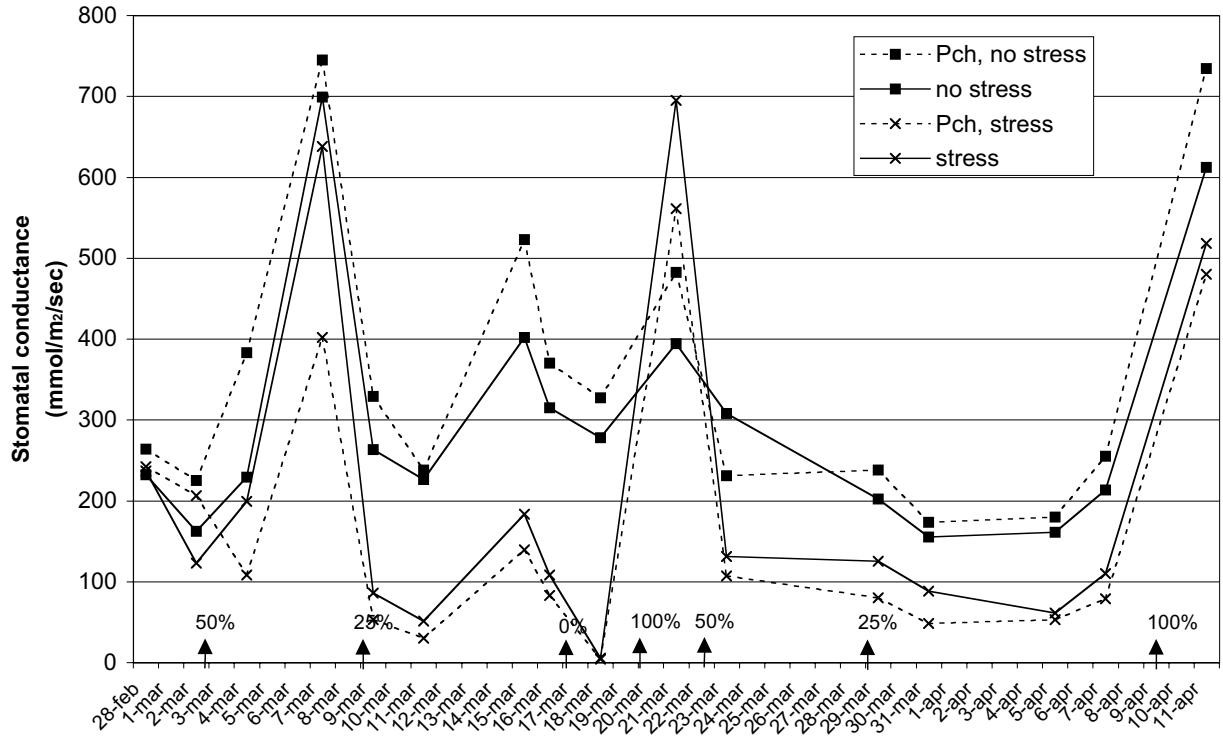

Fig. 5. Effects of water stress on stomatal conductance of infected and uninfected Zinfandel, Year 2, 2005.

significant. This can be clearly seen in the graphs (Fig. 8) as there is almost no infection effect on day 7, but a definite effect on days 9 and 13. At 6 pm, only the overall effect of stress and the interaction of date with stress were significant.

In addition to considering the measurements at particular times over several days, the rate of change (i.e. the slope between 9 am and 3 pm) was examined as a measure of the vines' response to stress. REML was used for estimation as before. The main effect of date was significant, i.e. on average the slopes get steeper over time. The main effect of stress was significant: i.e. on average over all days the slope of the unstressed vines is steeper. Since the interaction of stress and date was significant, it appears that as the plants became more stressed, the difference in the slopes between stressed and unstressed vines became greater. As for the significant interaction between date and infection, the slope of the uninfected vines remained constant while the slope of the infected vines became steeper. There is an interaction between stress and infection, evident in Fig. 8 where some of the treatment lines cross over each other.

In the case of leaf temperature, the effects of date and stress were highly significant, but infection was not (data not shown). There were no significant treatment differences for either shoot or root dry weights (data not shown).

Discussion

The results presented here clearly showed that *P. chlamydospora* infection interfered with the water relations of the grapevine, particularly under conditions of water deficit. In glasshouse experiments, the physiological responses to water stress of infected and uninfected Zinfandel were measured. Plants were subjected to a single steady stress (year 1), to a stress that steadily increased over time (year 2) and to the imposition of a short severe stress (year 2). Under all of these circumstances, grapevines infected with *P. chlamydospora* responded differently to comparable uninfected grapevines.

Measures of leaf water potential, stomatal conductance and leaf temperature are all commonly used indicators of water stress in grapevines

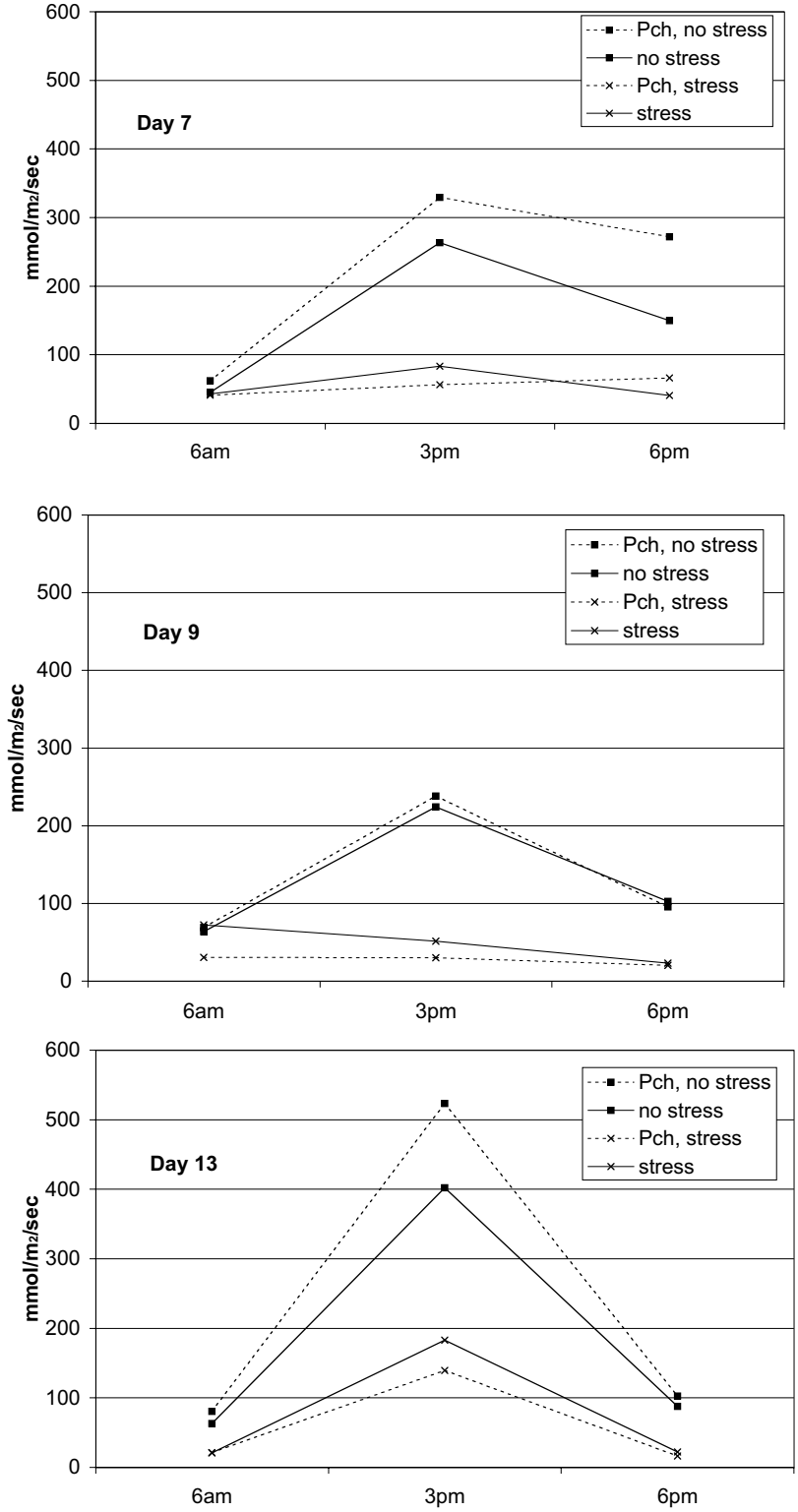

Fig. 6. Zinfandel, Year 2: 2005. Stomatal conductance measured at 6 am, 3 pm and 6 pm on days 7, 9 and 13 after stress treatments imposed.

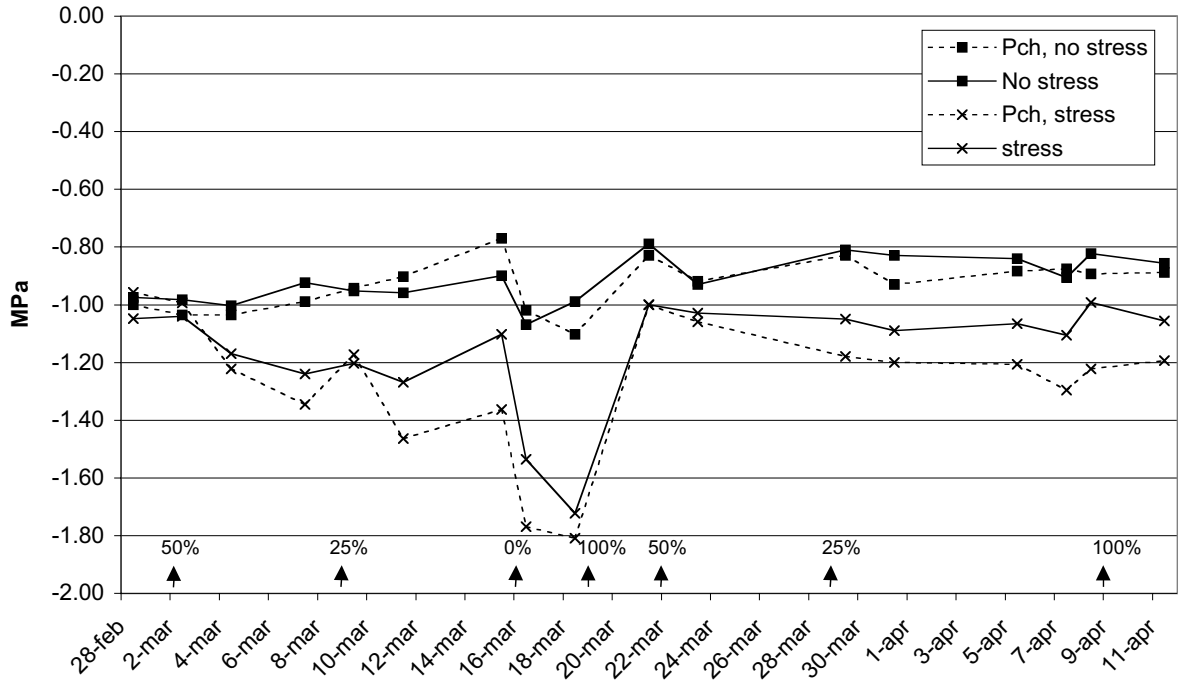

Fig. 7. Impact of *P. chlamydospora* infection on response of potted Zinfandel grapevines to water stress, as measured by leaf water potential at 3 pm, Year 2, 2005.

(Smart, 1974; Winkel and Rambal, 1993; Lovisolo and Schubert, 1998; Chone *et al.,* 2001; Escalona *et al.,* 2002; Williams and Araujo, 2002). Stomatal conductance and leaf water potential measurements were the most useful for differentiating between infected and uninfected plants. Leaf temperature differentiated between stressed and unstressed plants, but was not discriminating enough to detect more subtle differences.

Stomatal conductance was usually higher in infected plants than uninfected plants, including the unstressed treaments, indicating that infection interfered with normal stomatal regulation. A similar phenomenon was reported by Guimaraes and Stotz (2004) in scarlet runner beans (*Phaseolus coccineus*) infected with *Sclerotinia sclerotiorum*. They demonstrated that oxalic acid production by the fungus interfered with stomatal closure by upsetting the mechanisms controlling guard cell response. To our knowledge, though, no-one has yet investigated whether *P. chlamydospora* produces oxalic acid or not.

The impact of infection on water-stressed grapevines was most apparent when leaf water potential was measured. Under water stress, infected vines had consistently lower leaf water potentials indicating that they were more severely affected than uninfected vines subjected to the same stress. Infection was making it more difficult for the vine to get water to the leaves. This is not surprising in light of the disruption *P. chlamydospora* has been demonstrated to cause to xylem function (Edwards *et al*., 2007).

The diurnal measurements (taken throughout a single day) showed that the stress × infection interaction was more pronounced in the afternoon, when plant water demand was highest. The 6 am measurements showed that over time the infected plants were less able to recover overnight than the uninfected plants, and the 3 pm and 6 pm measurements showed that the infected plants were less able to cope with the additional burden of afternoon temperature. This was particularly noticeable in Year 2, when by day 13, the infected plants were clearly less able to cope with the water stress and did not recover overnight to the same level as the uninfected stressed plants.

In Australian viticulture, the current trend towards growing grapes for quality and the increased awareness of water as a limited resource

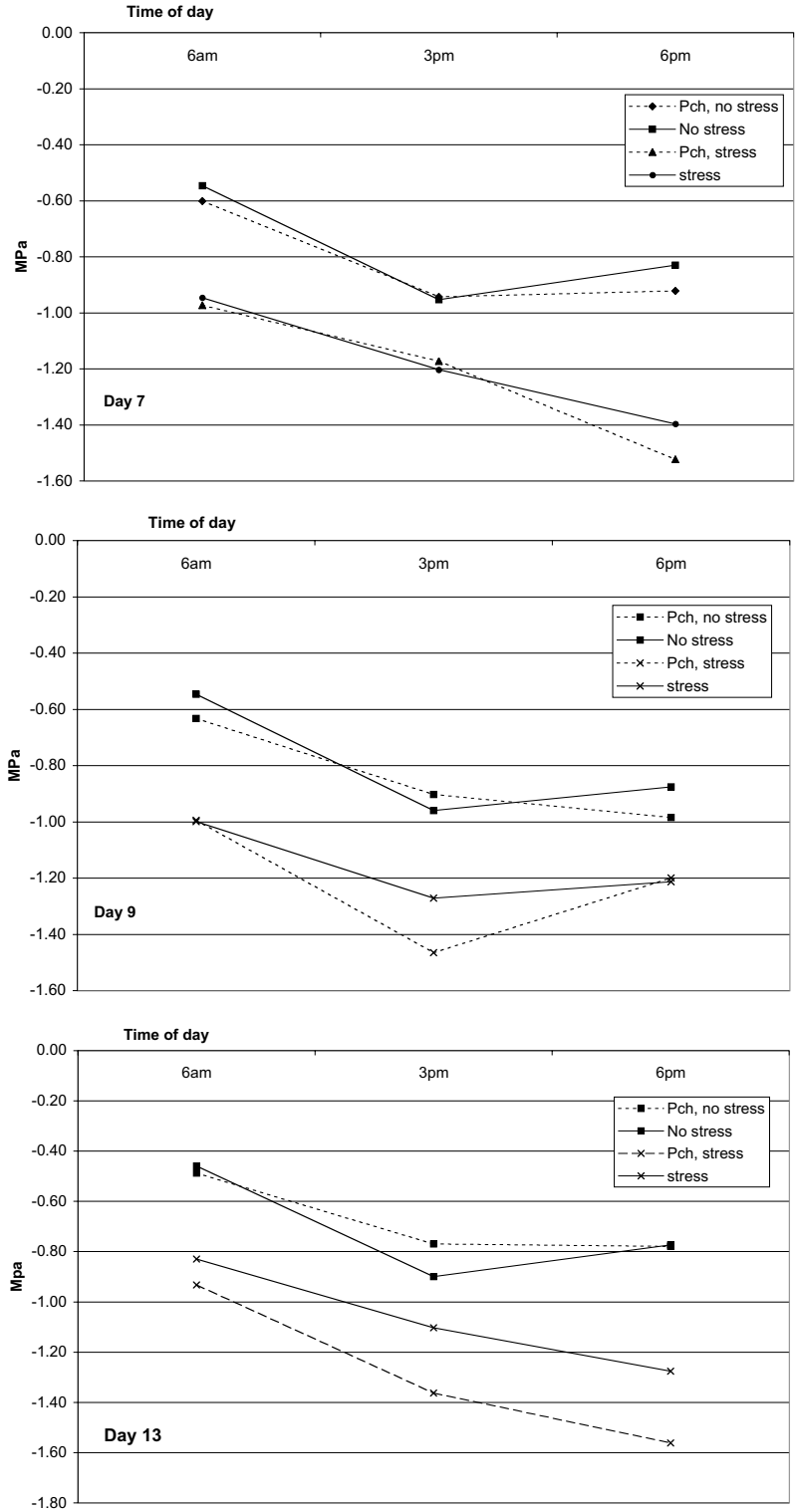

Fig. 8. Zinfandel, Year 2, 2005. Leaf water potential measured at 6 am, 3 pm and 6 pm on days 7, 9 and 13 after stress treatments imposed.

has meant there is considerable interest in applying irrigation scheduling which involves periods of water stress. Deficit irrigation such as regulated deficit irrigation and sustained deficit irrigation are being promoted, yet it is unknown how infected grapevines will respond to the challenges of increased water stress, and how best to manage them for long-term vineyard health and productivity.

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